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Gene Expression Profiling to Study Racial Differences after Heart Transplantation

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DISCLOSURES

Stanford University holds equity in CareDx.
David Hiller and James Yee are employees of CareDx, the manufacturer of AlloMap.
Jeffrey Teuteberg, Andrew Kao, and Mario Deng serve on the advisory board of CareDx.

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Abstract

Background—The basis for increased mortality after heart transplantation in African Americans and other non-Caucasian racial groups is poorly defined. We hypothesized that increased risk of adverse events is driven by biological factors. To test this hypothesis in the IMAGE study, we determined whether the event rate of the primary outcome of acute rejection, graft dysfunction, death, or re-transplantation varied by race as a function of calcineurin inhibitor levels and gene expression profile (GEP) scores.

Methods—We determined the event rate of the primary outcome, comparing racial groups, stratified by time post-transplant. Logistic regression was used to compute the relative risk across racial groups and linear modeling was used to measure the dependence of CNI levels and GEP score on race.

Results—In 580 patients followed for a median of 19 months, the incidence of the primary endpoint in African Americans, other non-Caucasians, and Caucasians was 18.3%, 22.2%, and 8.5%, respectively ($p<0.001$). There were small but significant correlations of race and tacrolimus trough levels to GEP score. Tacrolimus levels were similar between races. Of patients receiving tacrolimus, other non-Caucasians had higher GEP scores than the other racial groups. African American recipients demonstrated a unique decrease in expression of the FLT3 gene in response to higher tacrolimus levels.

Conclusions—African Americans and other non-Caucasian heart transplant recipients were 2.5–3 times more likely than Caucasians to experience outcome events in IMAGE. The increased risk of adverse outcomes may be partly due to the biology of the alloimmune response, which is less effectively inhibited at similar tacrolimus levels in minority racial groups.

Keywords

Heart transplantation; race; acute rejection; mortality

INTRODUCTION

Racial disparities in survival after solid organ transplantation were first recognized by Opelz and Terasaki in 1977, when large differences between African American and Caucasian recipients were identified after kidney transplantation.(1) Since then, multiple reports have demonstrated worse survival after heart transplantation in African American recipients, compared to other racial groups.(2–7)

Many reasons have been suggested to account for racial disparities in post-transplant outcomes. These include socioeconomic and educational factors,(2) access to high-quality medical care,(4) compliance, a higher prevalence of co-morbidities such as hypertension in African American recipients,(8) and fundamental immunologic differences.(9, 10)

The Invasive Monitoring Attenuation through Gene Expression (IMAGE) study,(11) which examined the clinical utility of monitoring for acute rejection after heart transplantation using peripheral blood gene-expression profiling, provided a unique opportunity to study the biology of racial differences in heart transplant outcomes. We sought to determine whether the incidence of acute rejection, graft dysfunction, death, or re-transplantation varied according to race, and to elucidate observed racial disparities in outcomes on the basis of immunosuppressive (calcineurin-inhibitor, CNI) drug levels and peripheral blood gene-expression patterns.

METHODS

Study design, patients, and procedures

The IMAGE study was a randomized trial conducted at 13 U.S. heart transplant centers between January 2005 and October 2009. The study design and procedures have been described previously.(11, 12) Adult heart transplant recipients, between 6 months and 5 years after transplant, were eligible for enrollment and were randomly assigned to undergo monitoring for rejection by means of gene-expression profiling (GEP) or routine endomyocardial biopsies (EMB). Patients assigned to the EMB group also had blood samples taken for GEP testing.

This study population (n = 602) was comprised of 12% African Americans and 6% other non- Caucasian participants, and compliance with medical therapies and rejection surveillance was closely monitored throughout the study period by trial coordinators. Data on medications and laboratory results enabled us to examine differences in immunosuppressive drug doses and CNI trough blood levels between racial groups. Finally, data on composite gene expression (AlloMap) scores and expression of each of the 11 genes comprising this test were available. These individual genes were originally selected to distinguish immune activation associated with acute rejection from a quiescent state. Analysis of IMAGE study data thereby enabled us to correlate clinical outcomes in different races with individual gene expression, which may provide insight into the biology of racial differences in transplant outcomes.

Gene-expression testing was performed with the use of the AlloMap test (CareDx), which evaluates expression levels of 11 informative genes that were shown in previous studies to distinguish between rejection and the absence of rejection.(13) Possible scores range from 0 to 40, with higher scores indicating a higher likelihood of histologic rejection. Patients were followed for a maximum of 24 months, until they died or until the study completion date, whichever occurred first.

Outcomes

The primary IMAGE trial outcome was the first occurrence of rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or re-transplantation. Rejection with hemodynamic compromise was defined as one or more of the following criteria: absolute drop in left ventricular ejection fraction (LVEF) ≤ 30%, proportional decrease in LVEF≥25% compared to the first study visit, cardiac index < 2 L/min/m2, and/or use of

inotropic drugs to support circulation at the time of the rejection episode. Death from any cause was considered as a secondary outcome.

Statistical Analysis

For the current analysis, 580 patients who had at least one Allomap score were studied. The patients were assigned to one of three groups based on self-identified race: Caucasian, African American, and other non-Caucasian, where the other non-Caucasian category includes predominantly Hispanic, Asian, and Pacific Islander populations. Within each group, study visits were classified by the time period post-transplant: 7 – 12 months, 13 – 36 months, and greater than 36 months. Demographics (age, racial group, CMV status, and number of rejections in the first year) of each racial group were assessed for the subset of patients with visits in each time period. CMV status was coded as a binary variable: 1 for donor positive/recipient negative and 0 otherwise. For demographic variables, each of three pairwise comparison between the racial groups were performed, using Tukey's test for continuous variables and Fisher's test with correction for multiple hypothesis testing via the Bonferroni method for discrete variables.

Racial differences in event rates—Event-free progression after study entry, within each racial group, was assessed using Cox modeling. Event rates and death rates for each racial group in each time period were calculated by dividing the total number of events observed during that time by the total length of time patients were followed.

Racial differences in calcineurin inhibitor levels—Only visits at which patients were taking a calcineurin inhibitor (CNI), either tacrolimus or cyclosporine, were included. CNI trough levels for each patient's visits falling within a particular time period were averaged to generate a summary for that patient and time period. Then, the patient averages were summarized across each racial group. When comparing drug levels between racial groups, pairwise comparisons, overall and within the 1 to 3 year time period, were performed using Tukey's test. We chose to focus on years 1 to 3 post-transplant since most study visits occurred during this time period.

Doses of prednisone greater than 20 mg per day and those administered for treatment of acute rejection were excluded from analyses of maintenance corticosteroid dosing.

Racial differences in gene expression profile scores—GEP scores for patient visits falling within a particular time period were also averaged to generate a summary for that patient and time period; patient averages were then summarized across each racial group. To compare GEP scores between racial groups, pairwise comparisons, overall and within the 1 to 3 year time period, were performed using Tukey's test.

To study the determinants of GEP score, a multivariate model was fit on patients treated with tacrolimus (n = 435), with tacrolimus trough level, prednisone dose, racial group, time post-transplant, age, sex, and CMV status as predictors. Univariate models were used to study associations between tacrolimus levels and each of the 11 gene components of the GEP score, both overall and within each racial group.

RESULTS

Patients

A total of 602 patients were enrolled in the IMAGE trial and 580 patients who had at least one GEP score were included in the current analysis. Caucasians comprised the majority of patients (78%), followed by African Americans (11%), and other non-Caucasians (11%). Baseline characteristics of the study population are presented in Table 1. Caucasian patients were older and were enrolled later (>1 year post-transplant) compared to the other racial groups. African American patients had a higher prevalence of diabetes, were less likely to be bridged to transplant with a left ventricular assist device (LVAD), and were more often randomized to the endomyocardial biopsy arm of the IMAGE study for rejection surveillance. Between-group comparisons of recipient sex, age, prevalence of CMV mismatch (CMV donor +/recipient −), and mean number of rejection events during the first year post-transplant revealed age as the only significant difference, with other non-Caucasians being younger than Caucasians. There were no significant differences in the percentage of patients treated with mycophenolate mofetil and maintenance corticosteroids between the three racial groups.

Racial differences in event rates

Comparison of freedom from the primary endpoint (Figure 1) demonstrates significant differences between the three racial categories ($p=0.001$ for Caucasian vs. African American; $p<0.001$ for Caucasian vs other non-Caucasian). Specifically, Caucasians had the highest probability of event-free progression [HR 0.87 (95% CI 0.81–0.91)] compared to African- Americans [HR 0.74 (0.57–0.85)], and other non-Caucasians [HR 0.70 (0.54–0.82)]. These results were consistent after adjusting for transplant center, recipient age and sex, use of pre-transplant LVAD, and CMV status (Supplemental Table 1).Table 2 summarizes the event rates of the composite outcome, as well as death, stratified by racial group and time post-transplant. The overall event rate and the death rate were, in general, higher in the African American and other non-Caucasian groups, compared to Caucasian transplant recipients.

Racial differences in calcineurin inhibitor levels

More patients were treated with tacrolimus (n = 435) than cyclosporine (n = 156). Tacrolimus was used more commonly for maintenance immunosuppression in Caucasian and African American recipients, whereas the choice of CNI was more balanced in other non-Caucasians (Table 2). A comparison of median calcineurin inhibitor trough levels demonstrated significantly higher cyclosporine levels and lower tacrolimus levels in other non-Caucasians compared to Caucasians. There were no significant differences in tacrolimus or cyclosporine trough levels between African American and Caucasian patients.

Racial differences in gene expression

We compared mean gene expression profile (GEP) scores between racial groups, as a function of time post-transplant and type of calcineurin inhibitor used for maintenance immunosuppression. As demonstrated in Table 2, GEP scores were overall similar between

the three racial groups, with the following exceptions: African Americans treated with cyclosporine had higher GEP scores than Caucasians and other non-Caucasians treated with cyclosporine; and, among tacrolimus-treated patients, other non-Caucasians had significantly higher GEP scores than the other two racial groups between 1 and 3 years post-transplant (p-value <0.001 for Caucasian vs other non-Caucasian; p-value 0.004 for African American vs other non-Caucasian).

We then examined determinants of the GEP score to identify factors that may contribute to differences in gene expression in the study population. In multivariate models (Supplemental Table 2), recipient race, transplant center, and CMV status were significantly correlated with the overall GEP score.

As a next step, we studied associations between tacrolimus levels and expression levels of the 11 individual genes that are used in the computation of the AlloMap ordinal score (Table 3). In the overall study cohort, increasing tacrolimus levels were associated with downregulation of MARCH8 (p<0.001) and WDR40A (p=0.004), genes that mediate hematopoiesis; and upregulation of ITGAM (p=0.005), a gene expressed by monocytes that mediates responsiveness to corticosteroids. These associations were mainly driven by changes in Caucasian recipients, who comprised a majority of the patient population. African American recipients demonstrated a unique decrease in expression of the FLT3 gene, which promotes B-cell and NK-cell proliferation, in response to higher tacrolimus levels (p=0.002) (Supplemental Table 3); however, this association was attenuated after adjusting for transplant center as well as recipient age, sex, pre-transplant LVAD, and CMV status (p=0.06).

Finally, we compared differences in individual gene expression between Caucasian and African American study participants. We found significantly increased expression of MARCH8 (p=0.001), and decreased expression of FLT3 (p=0.0002), and PDCD1 (p=0.002) in African Americans compared to Caucasians, even after correction for multiple hypothesis testing. FLT3 and MARCH8 expression were significant predictors of clinical events in African American subjects, even after adjusting for relevant covariates (Table 4).

DISCUSSION

We used data from the IMAGE trial, a prospective randomized clinical trial of peripheral blood gene-expression profiling versus endomyocardial biopsy for cardiac transplant rejection surveillance, to examine racial differences in post-transplant outcomes. Specifically, we used this unique data set to test the overall hypothesis that the increased risk of adverse events seen in non-Caucasian minority racial groups is driven by biological factors related to the alloimmune response.

As is consistent with prior studies,(2, 5) we found a higher incidence of the primary outcome (rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation) in African Americans and other non-Caucasians, compared to Caucasian trial participants. Previous work studying transplant outcomes have suggested that access to medical care and compliance with immunosuppressive medications may mediate the

discrepancies in outcomes seen between racial groups.(2, 4) Other studies, however, have shown that universal access to medical care (e.g. via the Veterans Administration healthcare system) does not eliminate racial disparities in transplant outcomes.(14) A clinical trial such as IMAGE therefore offers a unique opportunity to address this controversy: all patients were treated at major academic medical centers and were followed closely by medical staff and trial coordinators. Compliance was monitored in the context of trial participation, and immunosuppressive drug levels were followed closely. Indeed, few differences were seen in immunosuppressive drug levels between racial groups, suggesting that access to care and compliance were not major factors contributing to the disparity seen in the incidence of adverse post-transplant outcomes.

Immunologic differences between transplant recipients of distinct racial groups may play an important role. (6) Indeed, in tests of immune function such as mixed lymphocyte reactions and T helper:T suppressor cell ratios, African Americans were more likely than Caucasians to be strong responders.(8, 15, 16) Studies such as those published by Mehra in 2002,(17) in which African American heart transplant recipients treated with tacrolimus had greater freedom from rejection at 1-year post-transplant than those treated with cyclosporine suggest that more potent immunosuppressive therapies(18, 19) may attenuate the effects of racial differences in alloreactivity. Adding to this argument is the clinical observation that induction therapy with both monoclonal and polyclonal antibodies enhances outcomes in African American kidney transplant recipients,(20) who also appear to be more resistant than Caucasians to developing complications such as opportunistic infections.(21)

The immunological differences between races may result from a combination of influences. First, the genetic diversity of human leukocyte (HLA) antigens in African Americans, and the relative scarcity of African American donors, makes it likely that African Americans recipients will receive poorly HLA-matched hearts. Second, the expression of T-cell costimulatory molecules CD80 and CD86, which are necessary for T-lymphocyte activation during the alloimmune response, is higher in African Americans than Caucasians.(10) Finally, purified T cells from African American subjects demonstrate significantly increased proliferation to phytohemagglutinin compared to those from Caucasians.(10) Thus, racial variations in peripheral blood antigen presenting cell characteristics may contribute to disparities in allograft rejection rates.

Upon analyzing data from IMAGE, we found similar GEP scores between racial groups, stratified by time post-transplant. This result supports the theory that current standard immunosuppressive therapies, such as tacrolimus and mycophenolate mofetil, may diminish immunologic differences between races. Notable exceptions were the higher GEP scores seen in African American recipients treated with cyclosporine, which is considered to be less effective than tacrolimus in the prevention of heart transplant rejection in all races.(22, 23) This finding is consistent with the observation that African American patients in IMAGE who were treated with cyclosporine had a higher incidence of the primary outcome compared to those treated with tacrolimus (6/10 vs. 6/50, respectively, p=0.0015), and lends support to other clinical trials that have shown a higher incidence of rejection in African American recipients treated with cyclosporine.

In multivariate analysis, race was not a significant predictor of the GEP score. Tacrolimus level was a significant predictor, however. In univariate models, increasing tacrolimus levels were significantly associated with up- or down-regulation of individual genes in the 11-gene panel. We therefore conducted an exploratory analysis to examine the association of tacrolimus levels with expression of these individual genes, stratified by race. A decrease in FLT3 gene expression was uniquely seen in the African American sub-group, despite the relatively small size of this cohort, and was an independent predictor of clinical events in this group. This gene is primarily expressed in immature hematopoietic progenitor cells in the bone marrow, and the gene product (fms-related tyrosine kinase receptor or CD135) is a cell surface receptor for FLT3 ligand—a factor produced by bone marrow stem cells that acts in collaboration with growth factors and cytokines to stimulate the proliferation of stem cells, B-cells, dendritic cells, and NK-cells.(24–26) Although speculative, we hypothesize that downregulation of FLT3 in African American transplant recipients may contribute to the differential outcomes seen in this racial group, despite similar tacrolimus levels, corticosteroid doses, and overall GEP scores, when compared to Caucasians. The biology of FLT3 downregulation in this context requires further investigation, and may contribute to our understanding of the biology of racial differences in transplant outcomes.

This work has significant strengths and limitations. The association between African American race and poorer post-transplant outcomes, including an increased incidence of acute rejection and decreased survival, has long been recognized. Theories to account for this disparity have abounded, and have included socioeconomic and biological causes. Unfortunately, these etiologies cannot be further investigated in the context of registry analyses or retrospective observational studies. Within the framework of clinical trials such as IMAGE, which involve rigorous monitoring of study subjects, differences in access to care and follow-up are likely to be minimized. If present, race-based differences in outcomes are therefore more likely to reflect biological or genetic responses to immunosuppressive therapy, and such trials offer a unique opportunity to study these mechanisms. The close patient follow-up required by IMAGE, with rigorous documentation of drug doses and levels, adjudication of endomyocardial biopsy results and clinical events, and serial gene expression scores, enabled us to begin studying these biological differences. We were not, however, able to adjust for traditional markers of poor outcomes such as education and socioeconomic status in our analyses, as data on these variables were not collected in IMAGE. We were also unable to adjust for all patient covariates that may have accounted for differences in GEP scores between transplant centers. These include differences in racial distribution and demographic characteristics between centers, treatment strategies (e.g. induction immunosuppression, corticosteroid weaning), and patient characteristics (medical severity, degree of allosensitization). There were also significant between-center differences in timing of patient enrollment, as some centers started enrollment at 6-months post-transplant, whereas other centers enrolled patients at a median of 3-years post-transplant. An additional limitation is that the 11-gene GEP tool was developed in the CARGO (Cardiac Allograft Rejection Gene Expression Observational) study,(13) which enrolled 72% Caucasians. It is possible that this panel may be best suited to Caucasian recipients, and that other genes may be more relevant to non-Caucasian heart transplant recipients. We finally acknowledge that IMAGE study patients were felt to be at lower risk of rejection than the

heart transplant recipient population in general, as those with a history of antibody mediated rejection or recent acute cellular rejection were excluded from enrollment. This represents a selection bias of the original trial and therefore of any subsequent analyses.

While conducting studies such as this, it is important to recognize that race is an imperfect and ill-defined descriptor that encompasses important social, economic, and genetic differences. The African American race is highly genetically diverse, with historical roots in Western and Central Africa, the Caribbean, Central America, South America, and Europe. In fact, it is estimated that current African Americans inherit approximately 14–18% of their ancestry from Europeans,(27) thereby casting doubt on the utility of racial distinctions when examining clinical outcomes. Nevertheless, these terms are commonly used in clinical practice today and may help, albeit imperfectly, to tailor post-transplant therapies. In addition, we found a significantly higher incidence of the primary outcome in other non-Caucasians, compared to Caucasians. This group encompasses a diverse set of patients of Asian, Hispanic, and Hawaiian/Pacific Islander heritage, with a small number of patients representing each group; therefore, further analyses specific to this group were not attempted. Finally, as this was not a pre-specified analysis, there were significant differences between the three racial groups in terms of number of subjects enrolled, as well as age, time post-transplant, type of CNI therapy, and other potentially important confounders.

Conclusions

In summary, we used IMAGE trial data to study whether the increased incidence of adverse events after heart transplantation in African Americans and other non-Caucasians may be due to differences in the biology of the immune response. We found that, among patients receiving tacrolimus, other non-Caucasians had higher GEP scores than the other racial groups. African American recipients demonstrated a unique decrease in expression of the FLT3 gene in response to higher tacrolimus levels, and expression of this gene was an independent predictor of clinical events. Our work thus provides important preliminary data for future studies investigating racial differences in alloimmunity. These studies are important because, while potent immunosuppressive therapies have improved early survival in most racial groups, longer-term survival has improved in Caucasian but not in African American or Hispanic recipients, resulting in a marked disparity in outcomes in the current era.(28) Enhanced understanding of the biology of such racial disparities may enable us to appropriately tailor post-transplant therapies in order to improve outcomes for all patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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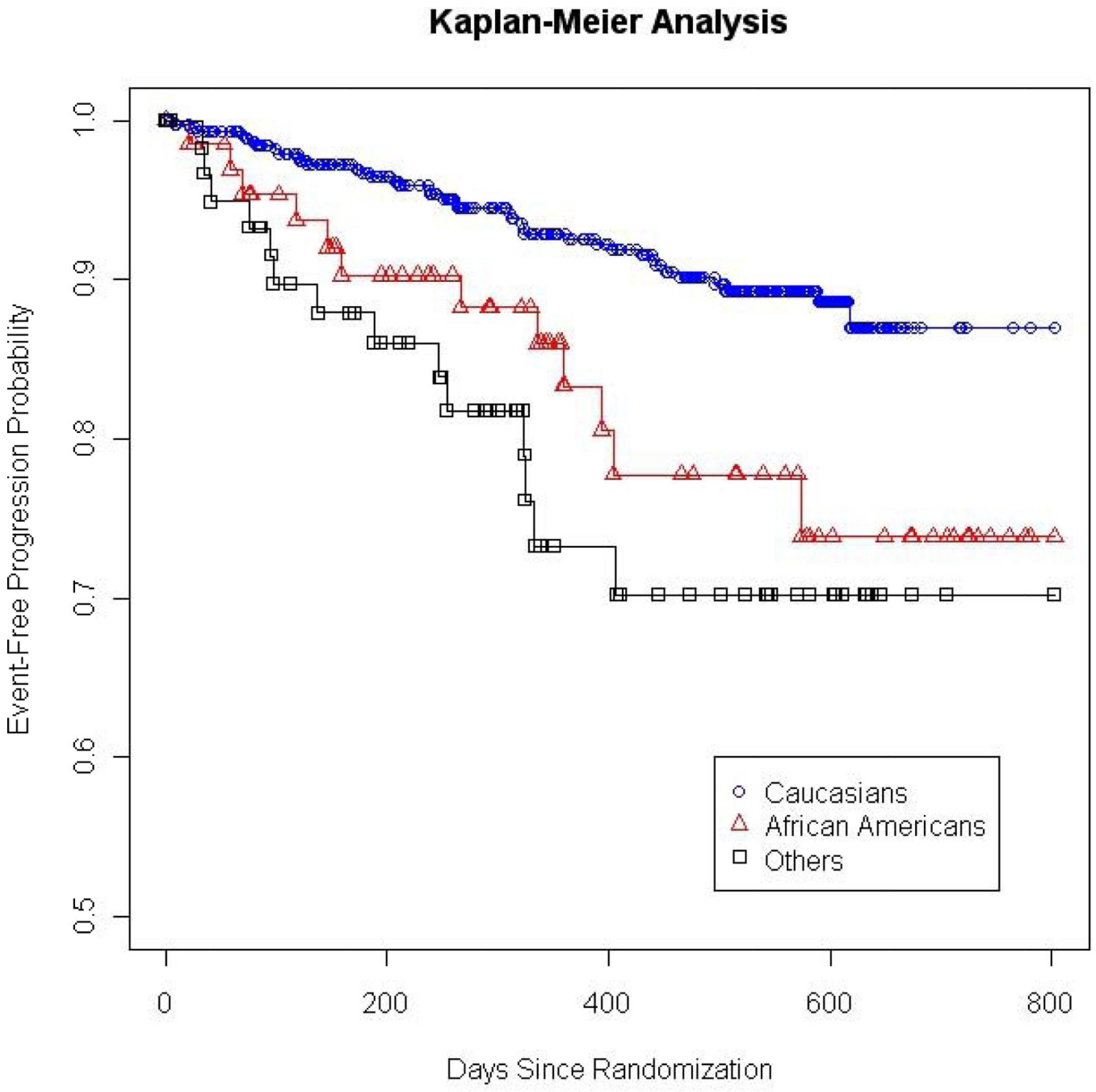

Figure 1.
Event-free progression of the primary outcome, by racial group

Table 1

Baseline Characteristics of the Study Population, by Racial Group

Characteristic	Caucasians (N=453)	African Americans (N=66)	Others (N=61)	p-value
Age -- yr				
Mean ± StdDev	55.2 ± 12.3	52.1 ± 12.2	47.2 ± 15.1	0.057
Range	18 – 78	22 – 74	19 – 71	
Male sex – no. (%)	375 (80.1)	50 (70.4)	48 (76.2)	0.08
Indication for cardiac transplantation – no. (%)				
Coronary artery disease	218 (46.6)	11 (15.5)	19 (30.2)	
Nonischemic cardiomyopathy	208 (44.4)	52 (73.2)	35 (55.6)	
Valvular heart disease	7 (1.5)	1 (1.4)	2 (3.2)	
Congenital heart disease	15 (3.2)	0 (0)	3 (4.8)	
Graft vasculopathy or retransplantation	2 (0.4)	1 (1.4)	1 (1.6)	
Other	3 (0.6)	1 (1.4)	1 (1.6)	
Interval between transplantation and randomization – no. (%)				
6 – 12 months	55 (11.8)	16 (22.5)	14 (22.2)	0.01
13 – 36 months	317 (67.7)	42 (59.2)	39 (61.9)	0.32
37 – 60 months	80 (17.1)	8 (11.3)	8 (12.7)	0.3
Cytomegalovirus status – no. (%)				
Donor and recipient positive	170 (36.3)	32 (45.1)	27 (42.9)	0.1
Donor and recipient negative	81 (17.3)	6 (8.5)	2 (3.2)	0.08
Donor positive and recipient negative	107 (22.9)	11 (15.5)	11 (17.5)	0.27
Donor negative and recipient positive	74 (15.8)	14 (19.7)	18 (28.6)	0.38
Unknown	21 (4.5)	3 (4.2)	3 (4.8)	
Use of ventricular assist device before transplantation – no. (%)	91 (19.4)	4 (5.6)	14 (22.2)	0.004
Induction therapy – no. (%)				
Any	260 (55.6)	40 (56.3)	37 (58.7)	0.69
Muromonab-CD3	4 (0.9)	0 (0)	5 (7.9)	
Antithymocyte globulin	64 (13.7)	4 (5.6)	5 (7.9)	
Basiliximab	79 (16.9)	19 (26.8)	5 (7.9)	
Daclizumab	85 (18.2)	12 (16.9)	23 (36.5)	
Alemtuzumab	19 (4.1)	4 (5.6)	0 (0)	
Other	43 (9.2)	3 (4.2)	10 (15.9)	
Immunosuppressive therapy – no. (%)				
Cyclosporine	120 (25.6)	10 (14.1)	26 (41.3)	0.05
Tacrolimus	338 (72.2)	57 (80.3)	38 (60.3)	0.04
Mycophenolate mofetil or mycophenolic acid	374 (79.9)	59 (83.1)	47 (74.6)	0.21
Azathioprine	35 (7.5)	3 (4.2)	3 (4.8)	0.46
Sirolimus	93 (19.9)	9 (12.7)	15 (23.8)	0.25
Prednisone	190 (40.6)	33 (46.5)	37 (58.7)	0.23
Medical history after transplantation – no. (%)				

Characteristic	Caucasians (N=453)	African Americans (N=66)	Others (N=61)	p-value
Hypertension treated with medication	386 (82.5)	57 (80.3)	46 (73)	1
Diabetes mellitus treated with medication	157 (33.5)	34 (47.9)	29 (46)	0.009
Renal insufficiency	235 (50.2)	39 (54.9)	21 (33.3)	0.29
Lipid-lowering drug prescribed	421 (90)	59 (83.1)	57 (90.5)	0.32
Cancer	74 (15.8)	6 (8.5)	4 (6.3)	0.15
Left ventricular ejection fraction at first study visit (%)				
Mean ± StdDev	63.4 ± 6.2	62.3 ± 6.5	63.7 ± 4.6	0.19
Study arm				
Gene expression profiling	232 (49.6)	24 (33.8)	36 (57.1)	0.03
Endomyocardial biopsy	221 (47.2)	42 (59.2)	25 (39.7)	0.03

Table 2

Comparison of overall event rates, death rates, calcineurin inhibitor levels, gene expression scores, and corticosteroid use by racial group and time post-transplant

Group	Months post-transplant	Event rate (events/patient/year)	Death rate (deaths/patient/year)	Tacrolimus median trough (N)	Cyclosporine median trough (N)	Mean GEP score Tacrolimus	Mean GEP score Cyclosporine	% patients on prednisone
Caucasian	7–12	0.09	0.00	8.8 (38)	167 (9)	28.8	26.1	88%
	13–36	0.08	0.02	8.2 (260)	144 (86)	30.0	30.1	34%
	> 36	0.07	0.02	7.0 (163)	118 (75)	30.0	30.0	23%
African American	7–12	0.27	0.27	9.3 (12)	141 (3)	30.5	32.2	64%
	13–36	0.20	0.04	8.4 (48)	120 (7)	29.8	32.2	33%
	> 36	0.30	0.10	7.1 (18)	111 (5)	29.4	30.5	42%
Other Non-Caucasian								
	7–12	1.30	0.43	8.2 (5)	183 (7)	29.7	28.5	83%
	13–36	0.27	0.08	7.0 (30)	173 (20)	31.8	29.4	45%
	> 36	0.16	0.04	5.7 (18)	122 (10)	29.6	28.0	26%

GEP: gene expression profiling

Table 3

Genes comprising the gene expression profile score, with site of expression and putative function

AlloMap Genes	Predominant Source of Expression in Blood	Role in Immune Activation and Rejection
ILIR2, ITGAM, FLT3	Monocytes	Steroid response
MARCH8, WDR40A	Reticulocytes	Proliferation and mobilization of erythrocytes
PF4, C6orf25	Platelets	Platelet activation
RHOU	T cells and monocytes	unknown
PDCD1	T cells	T cell activation
ITGA4	T cells	T cell migration
SEMA7A	T cells, B cells, and immature neutrophils	unknown

Table 4

Multivariate model examining predictors of clinical event in African Americans

Predictor	p-value
FLT3 Expression	0.005
MARCH8 Expression	0.002
Transplant center	1 center $p < 0.05$
Age	0.04
Sex	0.05
LVAD Bridge to Transplant	0.03
Study Arm (GEP or EMB)	0.04
Prednisone use	< 0.001
Tacrolimus trough level (per 1 ng/ml increase)	0.64
CMV Status (donor +/recipient −)	0.003
Days post transplant	0.004

LVAD: left ventricular assist device; CMV: cytomegalovirus
GEP: gene expression profiling; EMB: endomyocardial biopsy
CMV: cytomegalovirus